

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 09/312,351

Applicants : **Jon A. Wolff et al.**

Filed : 05/14/1999

Art Unit : 1621

Examiner : **Valenrod, Yevgeny**

Docket No. : **Mirus.006**

For: **A Compound Containing a Labile Disulfide Bond**

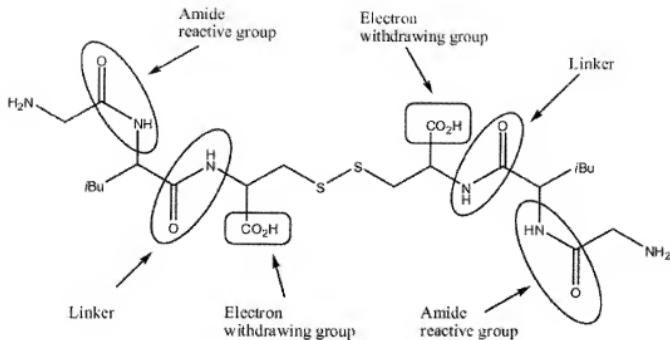
Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

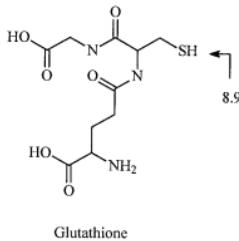
Dear Sir:

I, Dr. Sean D. Monahan, hereby declare as follows:

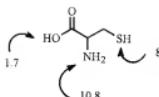
1. I have a Doctorate in Organic Chemistry from the University of Wisconsin, Madison.
2. I am familiar with the above captioned application.
3. The Action states that the Abderhalden compound (shown below, with groups labeled by the Examiner) meets all structural limitation of claims 7, 8, 19 and 20 of the above captioned application. The Abderhalden structure does contain a disulfide bond. However, the disulfide bond described in this example would *not* be cleaved more rapidly than oxidized glutathione in physiological conditions, because the pKa's of the constituent thiols of the Abderhalden compound are greater than glutathione thiol pKa.



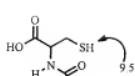
Glutathione is an N-acyl cysteine amide with a thiol pKa of 8.9 (see below, thiol pKA listed¹). With glutathione and other thiol-containing compounds, substituents proximal to the thiol affect the pKa of the thiol¹.



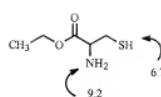
The Abderhalden compound is an example of a disulfide derived from an N-acylated cysteine. Glutathione can be considered an N-acylated cysteine also. Acylation of cysteine increases the pKa of the thiol from 8.3 (cysteine pKa) to 9.5 (N-acetyl cysteine). Penicillamine, a dimethyl derivative shows a similar trend. Amidation or esterification of the cysteine carboxylic acid on the other hand, lowers the pKa of the thiol (6.7 for cysteine ethyl ester).



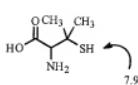
Cysteine



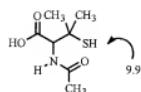
N-Acetyl Cysteine



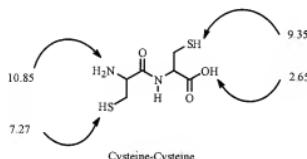
Cysteine Ethyl Ester



Penicillamine



N-Acetyl Penicillamine



Cysteine-Cysteine

As another example, for cysteine-cysteine, the pKa of the thiol on the N-terminal amino acid is 7.27, whereas the pKa of the C-terminus cysteine thiol is 9.35 again indicating that amidation of the cysteine carboxylic acid decreases the pKa of the thiol (relative to cysteine). Glutathione (an N-acyl cysteine amide) has the cysteine carboxylic acid amidated, lowering the pKa of the thiol. The combination of the amide group (linker in the Examiners' drawing) with the carboxylic acid group of Abderhalden compound results in a compound that is readily recognizable in the art as having a thiol pKa that is **greater** than the thiol of glutathione, contrary to the limitation of a disulfide bond being cleaved more rapidly than oxidized glutathione.

1- pKa values taken from *Handbook of Biochemistry and Molecular Biology*
From the chapter on Ionization Constants of Acids and Bases by Jencks, W. P. and Regenstein, J.

Cumulative Series index for CRC Handbook of Biochemistry and Molecular Biology, Cleveland, Ohio, CRC Press, 1977, 3rd ed., Gerald D. Fasman ed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.


Dr. Sean D. Monahan

8/13/08

date